

Sorting by pools

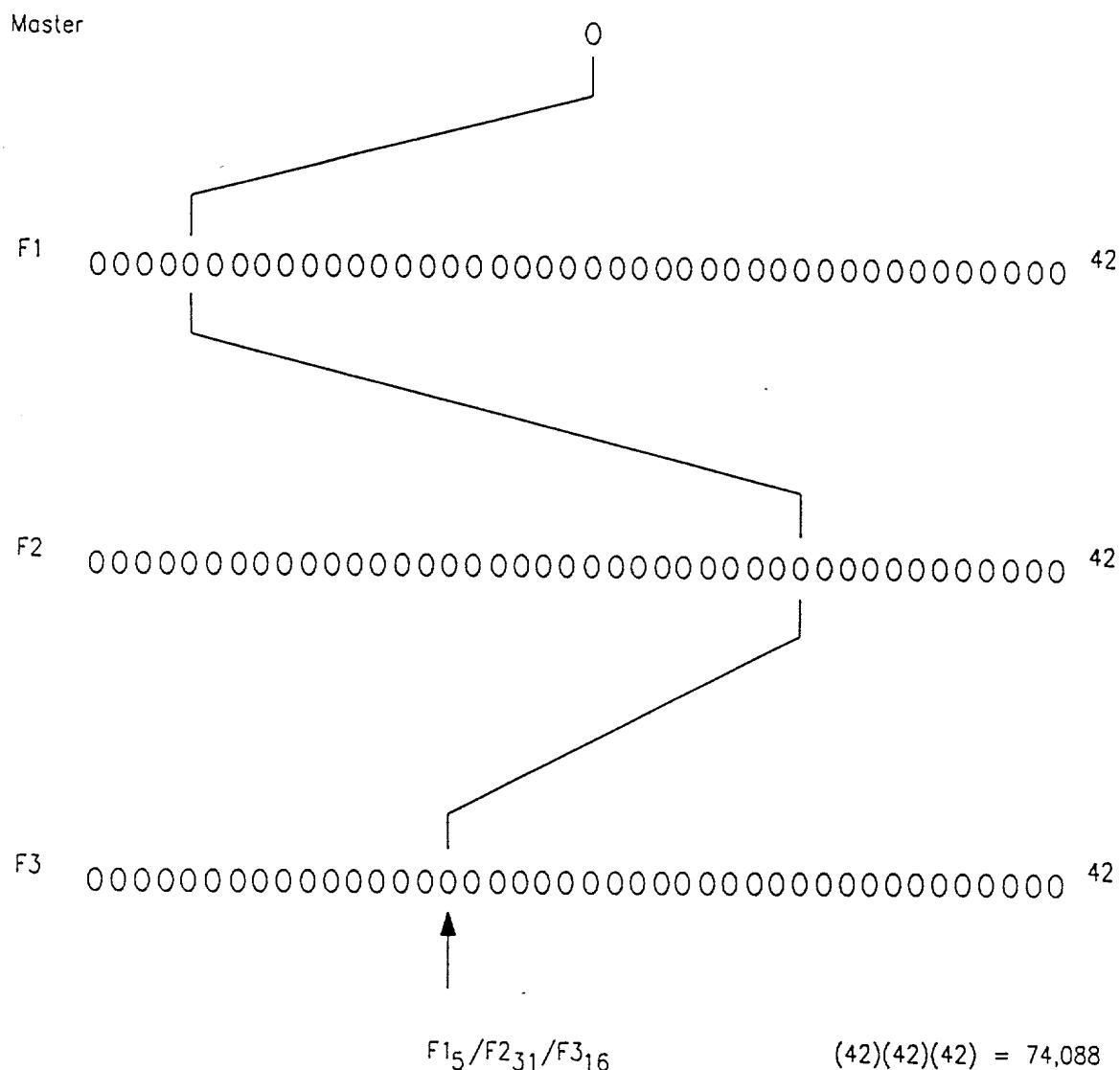


FIG. I

Sorting by pools: Decreasing pool diversities

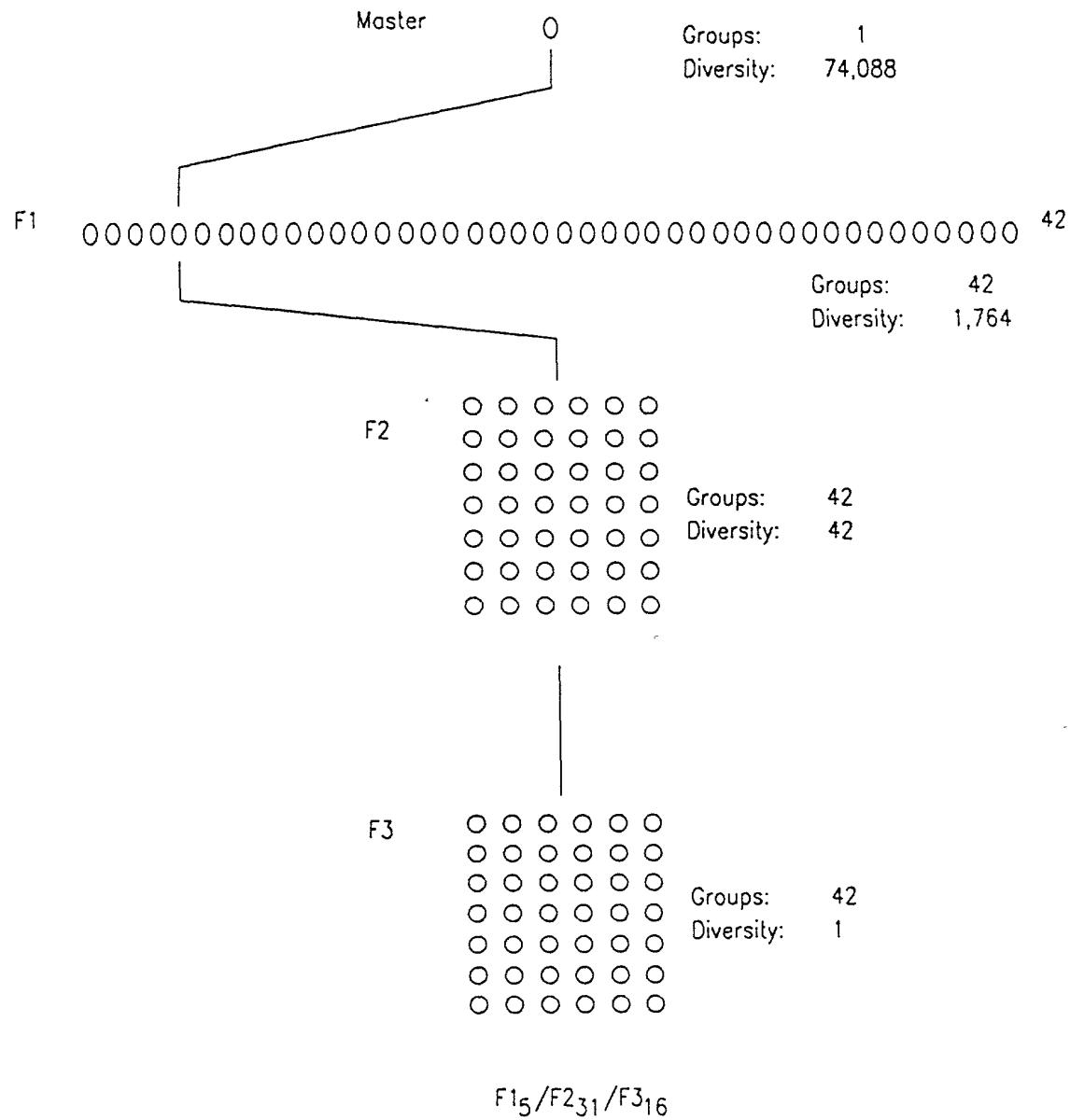


FIG. 2

Sorting by pools: Screening large diversity libraries

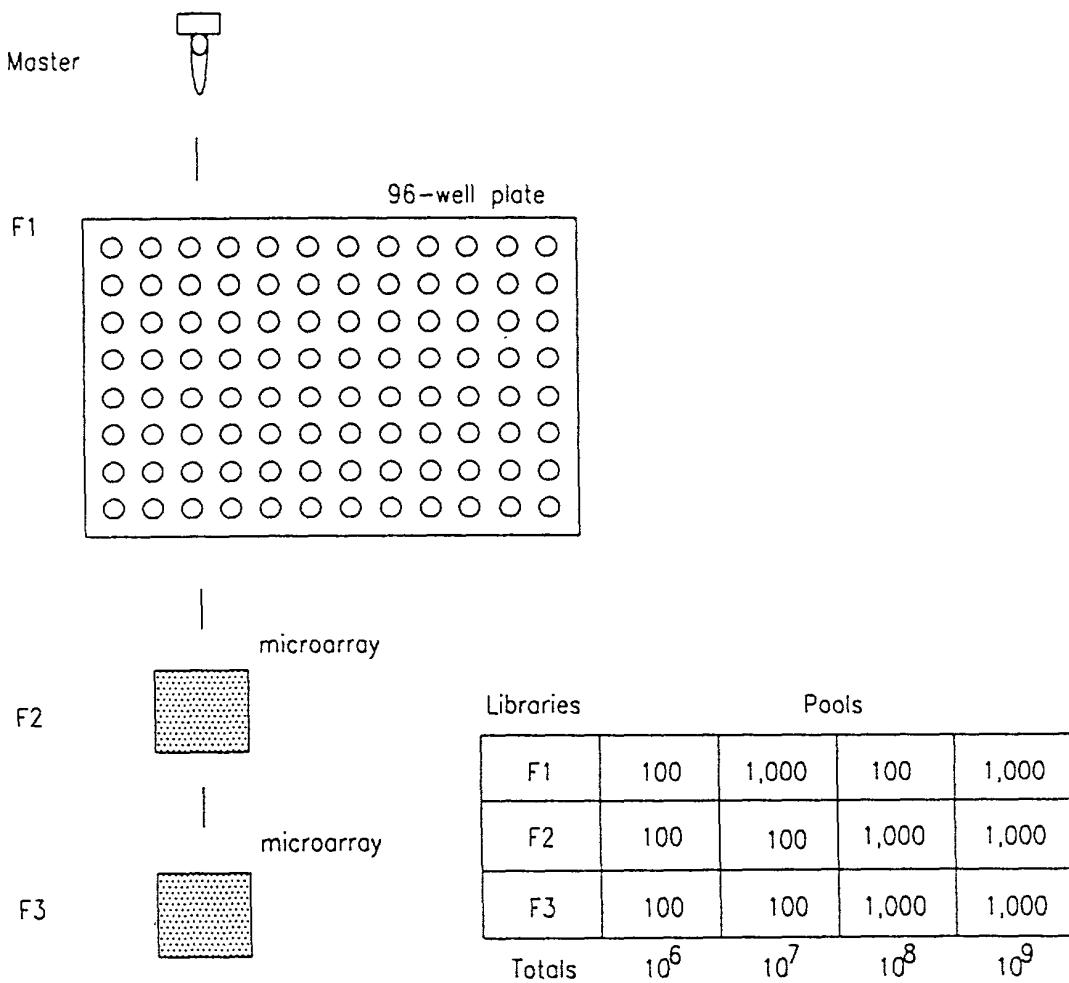


FIG. 3

Searching a mutation library

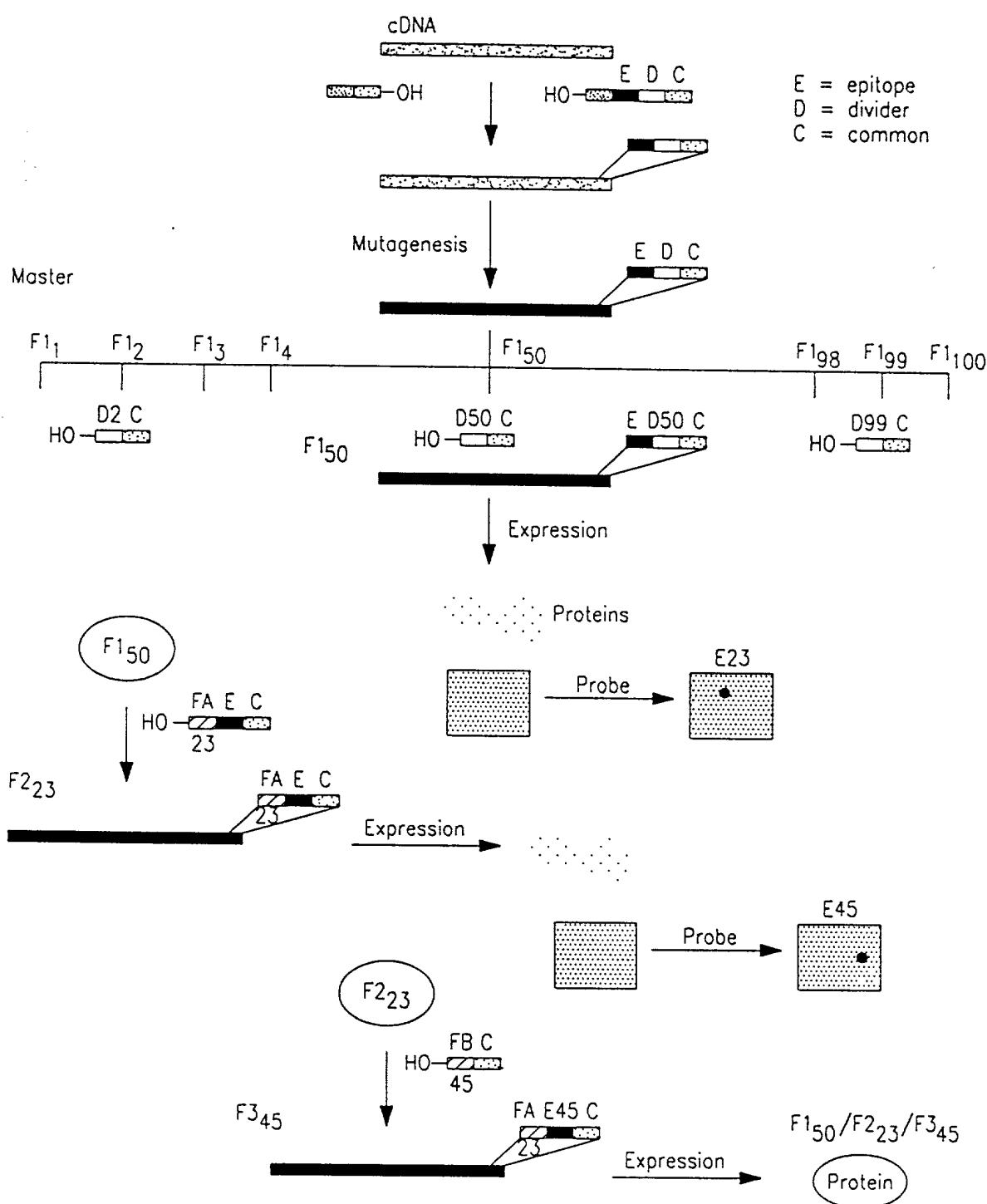


FIG. 4

Making a recombinant antibody library

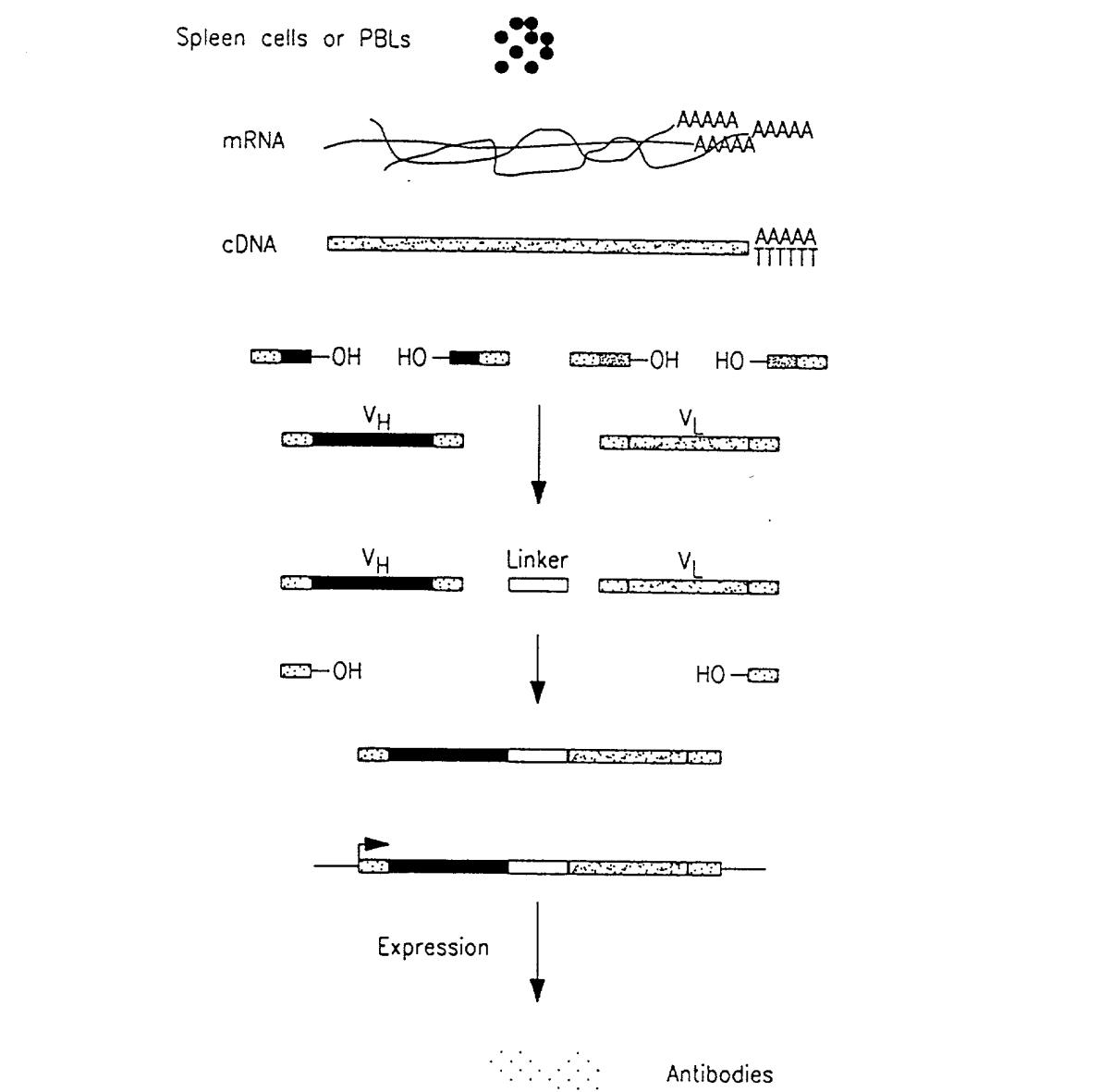
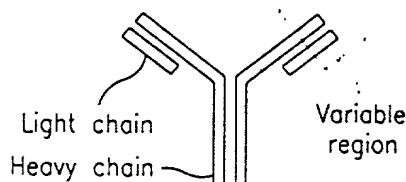
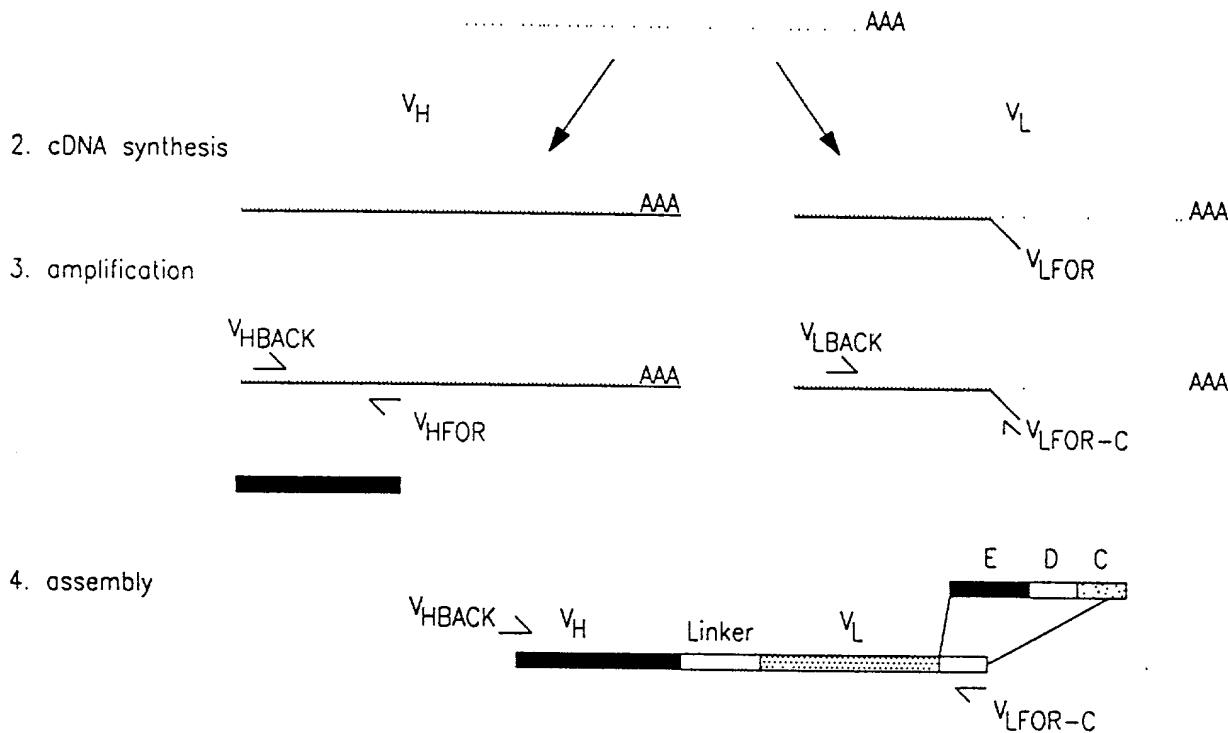


FIG. 5

Creating the master antibody library: Primer incorporation

1. mRNA purification from spleen or PBLs



V_H Primers		V_L Primers	
Oligo dT	$HO-5' \text{---} TTTTTTTT(T)_n-3'$	V_L FOR	$HO-5' \text{---} J_{kappa} \text{ for } E \text{ --- D --- C }-3'$
V_H BACK	$5' \text{ --- } V_H \text{ back } \text{ (cross-hatched box) } \text{ --- } OH-3'$	V_L BACK	$5' \text{ --- } V_{kappa} \text{ back } \text{ (cross-hatched box) } \text{ --- } OH-3'$
V_H FOR	$OH-5' \text{ --- } J_H \text{ for } \text{ (cross-hatched box) }-3'$	V_L FOR-C	$HO-5' \text{ --- } C-3'$

FIG. 6

Creating the master antibody library: Linker addition

1. mRNA purification from spleen or PBLS

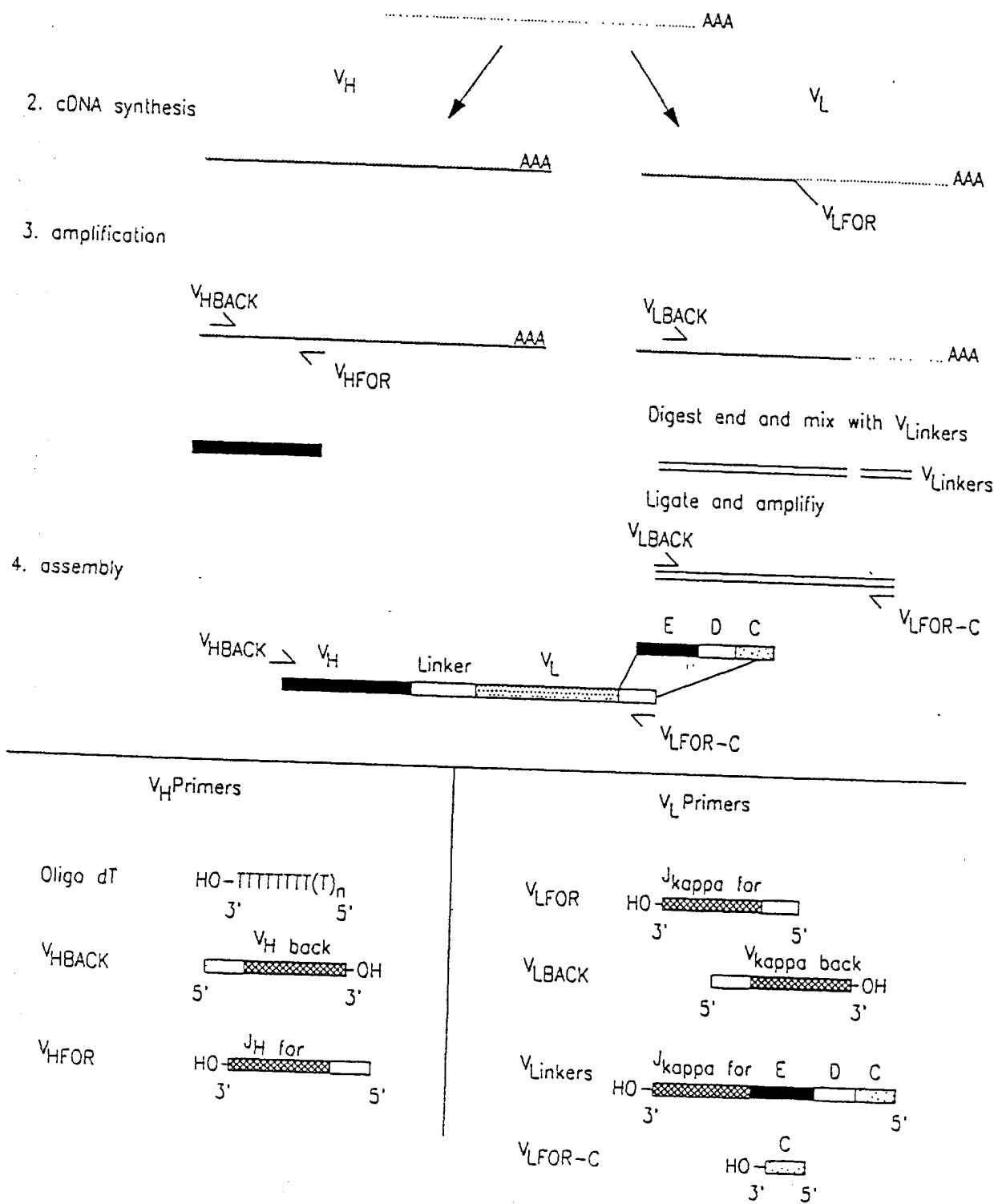


FIG. 7

Searching a recombinant antibody library

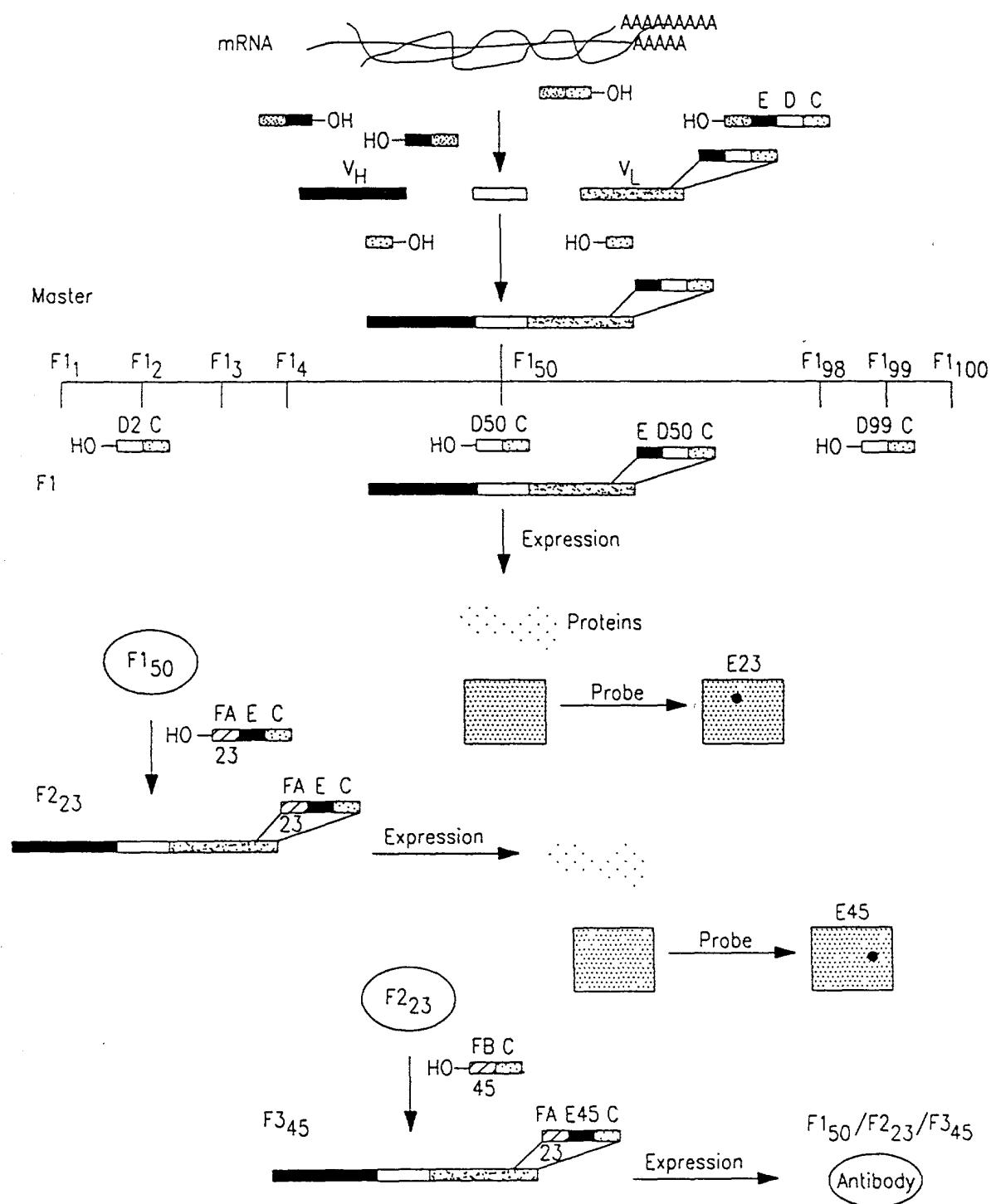
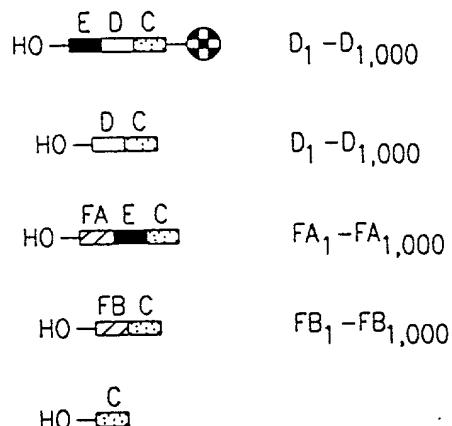


FIG. 8

Physical elements to include in the kits and combinations

- *Anti-tag Arrays™*

- Primer sets

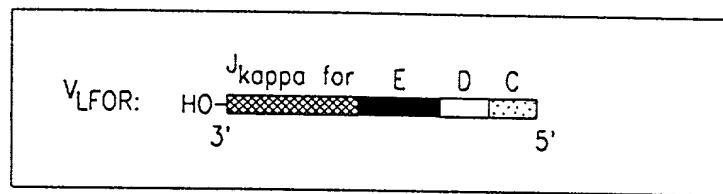


- Readers

- Software

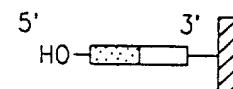
FIG. 9

Making the V₁FOR primers: Solid phase synthesis

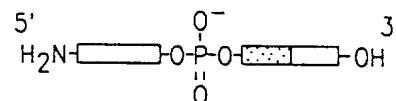


λ kappa for Epitope D Common

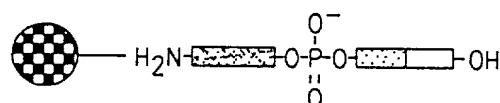
1. Synthesize oligo on solid support



2. Add aminolink prior to cleavage



3. Couple to tosyl activated magnetic beads



4. Extended by hybridizing with DNA patch and ligating

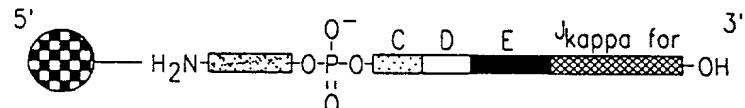
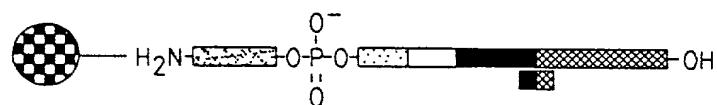
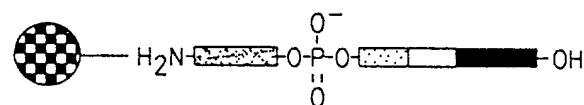
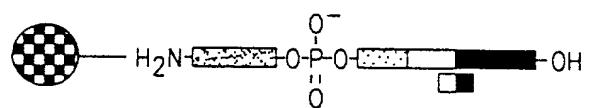
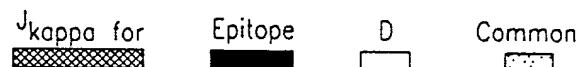
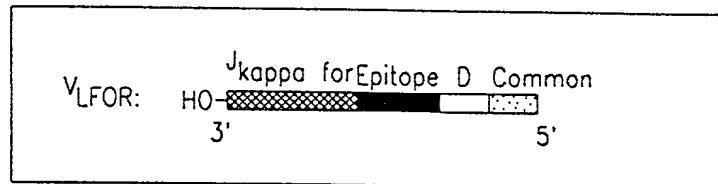


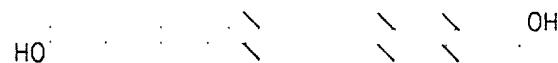
FIG. 10

Making the V_{LFOR} primers: Overlapping hybridization



- Synthesize 4,028 different oligos:
(26 for J κ for ; 2,000 for Epitope, 2,000 for D; 2 for Common

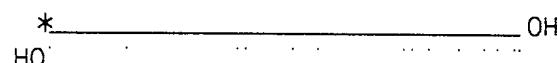
2. Assemble oligos for + and - strands of the different regions



3. Ligase the assembled oligos



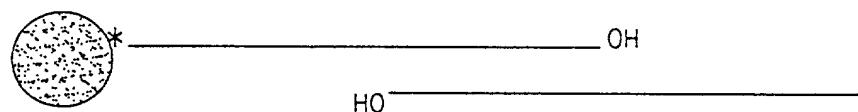
4. 1st strand synthesis with biotinylated primer



- 2nd strand synthesis with non-biotinylated primer



6. Bind to avidin coated magnetic beads and then denature



7. Purify non-biotinylated ssDNA

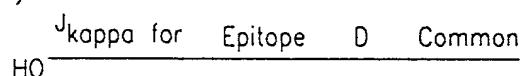


FIG. I

Building the collection of antibody/tag pairs: Hybridoma screening

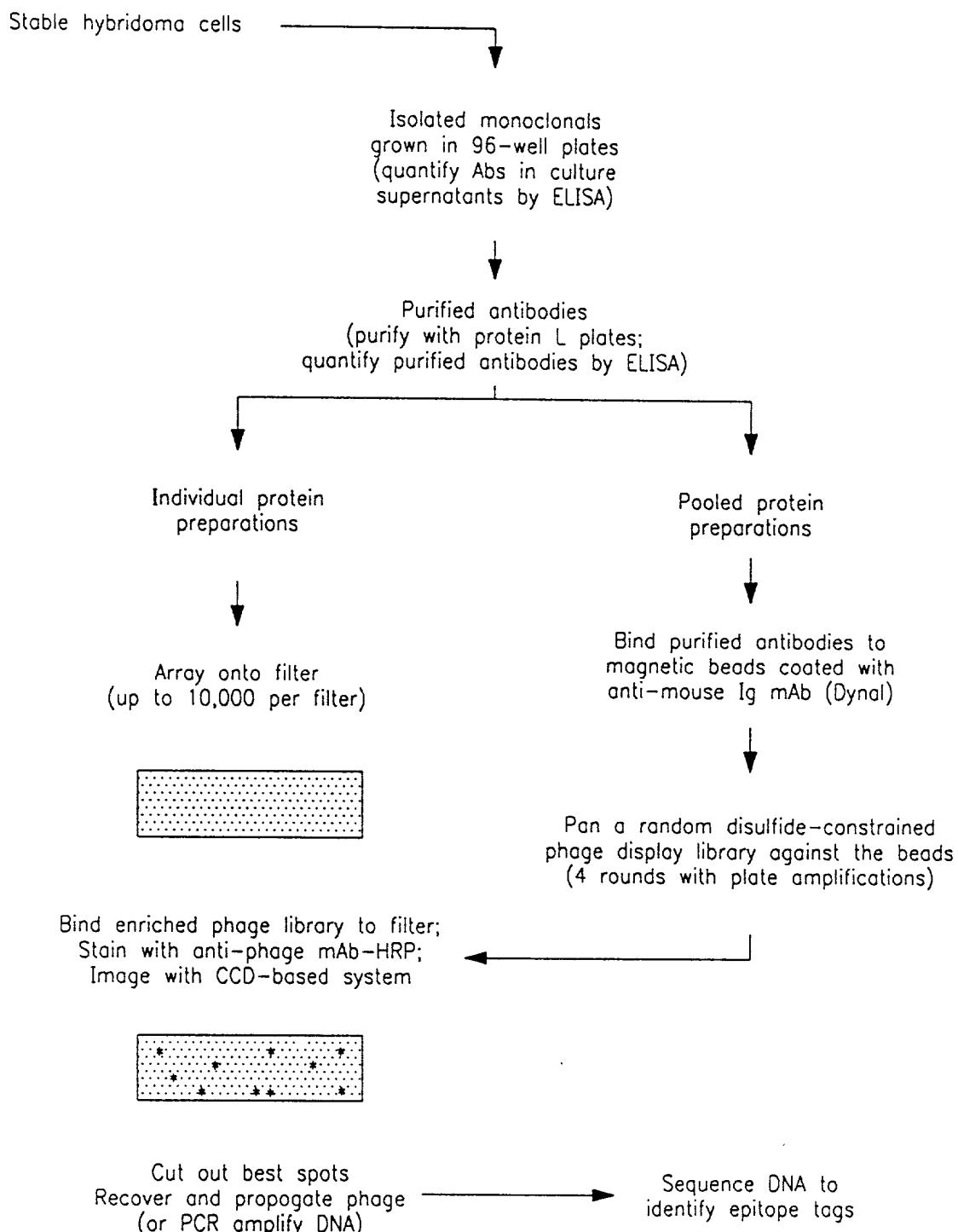


FIG. 12

Title: COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUGHPUT SCREENING.

Applicant: Ault-Riche *et al.*
Serial No. 09/910,120 Filed: July 18, 2001
Our Docket No. 25885-1751

Table 3 Primers for PCR Amplification of Human Antibody Variable Regions (V genes)

1. V gene primary PCR

A. Human VH back primers (sense)

HuVH1aBACK	5'-CAG GTG CAG CTG GTG CAG TCT GG-3'
HuVH2aBACK	5'-CAG GTC AAC TTA AGG GAG TCT GG-3'
HuVH3aBACK	5'-GAG GTG CAG CTG GTG GAG TCT GG-3'
HuVH4aBACK	5'-CAG GTG CAG CTG CAG GAG TCG GG-3'
HuVH5aBACK	5'-GAG GTG CAG CTG TTG CAG TCT GC-3'
HuVH6aBACK	5'-CAG GTA CAG CTG CAG TCA GG-3'

B. Human JH forward primers (anti-sense)

HuJH1-2FOR	5'-TGA GGA GAC GGT GAC CAG GGT GCC-3'
HuJH3FOR	5'-TGA AGA GAC GGT GAC CAT TGT CCC-3'
HuJH4-5FOR	5'-TGA GGA GAC GGT GAC CAG GGT TCC-3'
HuJH6FOR	5'-TGA GGA GAC GGT GAC CGT GGT CCC-3'

C. Human V kappa back primers (sense)

HuVk1aBACK	5'-GAC ATC CAG ATG ACC CAG TCT CC-3'
HuVk2aBACK	5'-GAT GTT GTG ATG ACT CAG TCT CC-3'
HuVk3aBACK	5'-GAA ATT GTG TTG ACG CAG TCT CC-3'
HuVk4aBACK	5'-GAC ATC GTG ATG ACC CAG TCT CC-3'
HuVk5aBACK	5'-GAA ACG ACA CTC ACG CAG TCT CC-3'
HuVk6aBACK	5'-GAA ATT GTG CTG ACT CAG TCT CC-3'

C. Human V lambda back primers (sense)

HuVλ1BACK	5'-CAG TCT GTG TTG ACG CAG CCG CC-3'
HuVλ2BACK	5'-CAG TCT GCC CTG ACT CAG CCT GC-3'
HuVλ3aBACK	5'-TCC TAT GTG CTG ACT CAG CCA CC-3'
HuVλ3bBACK	5'-TCT TCT GAG CTG ACT CAG GAC CC-3'
HuVλ4BACK	5'-CAC GTT ATA CTG ACT CAA CCG CC-3'
HuVλ5BACK	5'-CAG GCT GTG CTC ACT CAG CCG TC-3'
HuVλ6BACK	5'-AAT TTT ATG CTG ACT CAG CCC CA-3'

D. Human J kappa forward primers (anti-sense)

HuJκ1FOR	5'-ACG TTT GAT TTC CAC CTT GGT CCC-3'
HuJκ2FOR	5'-ACG TTT GAT CTC CAG CTT GGT CCC-3'
HuJκ3FOR	5'-ACG TTT GAT ATC CAC TTT GGT CCC-3'
HuJκ4FOR	5'-ACG TTT GAT CTC CAC CTT GGT CCC-3'
HuJκ5FOR	5'-ACG TTT AAT CTC CAG TCG TGT CCC-3'

D. Human J. lambda forward primers (anti-sense)

HuJλ1FOR	5'-ACC TAG GAC GGT GAC CTT GGT CCC-3'
HuJλ2-3FOR	5'-ACC TAG GAC GGT CAG CTT GGT CCC-3'
HuJλ4-5FOR	5'-ACC TAA AAC GGT GAG CTG GGT CCC-3'

FIG. 13A

Title: COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUGHPUT SCREENING.

Applicant: Ault-Riche *et al.*
Serial No. 09/910,120 Filed: July 18, 2001
Our Docket No.: 25885-1751

2. Linker fragment PCR

F. Reverse JH for scFv linker (sense)

—FR4 heavy ——————+—linker
 RHuJH1-2 5'-GC ACC CTG GTC ACC GTC TCC TCA GGT GG-3'
 RHuJH3 5'-GG ACA ATG GTC ACC GTC TCT TCA GGT GG-3'
 RHuJH4-5 5'-GA ACC CTG GTC ACC GTC TCC TCA GGT GG-3'
 RHuJH6 5'-GG ACC ACG GTC ACC GTC TCC TCA GGT GG-3'

F. Reverse Vk for scFv linker (anti-sense)

—FR1 light ——————+—linker
 RHuV_k1aBACKFv 5'-GG AGA CTG GGT CAT CTG GAT GTC CGA TCC GCC-3'
 RHuV_k2aBACKFv 5'-GG AGA CTG AGT CAT CAC AAC ATC CGA TCC GCC-3'
 RHuV_k3aBACKFv 5'-GG AGA CTG CGT CAA CAC AAT TTC CGA TCC GCC-3'
 RHuV_k4aBACKFv 5'-GG AGA CTG GGT CAT CAC GAT GTC CGA TCC GCC-3'
 RHuV_k5aBACKFv 5'-GG AGA CTG CGT GAG TGT CGT TTC CGA TCC GCC-3'
 RHuV_k6aBACKFv 5'-GG AGA CTG AGT CAG CAC AAT TTC CGA TCC GCC-3'

F. Reverse V_λ for scFv linker (anti-sense)

—FR1 light ——————+—linker
 RHuV_λBACK1Fv 5'-GG CGG CTG CGT CAA CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
 RHuV_λBACK2Fv 5'-GC AGG CTG AGT CAG AGC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
 RHuV_λBACK3aFv 5'-GG TGG CTG AGT CAG CAC ATA GGA CGA TCC GCC ACC GCC AGA G-3'
 RHuV_λBACK3bFv 5'-GG GTC CTG AGT CAG CTC AGA AGA CGA TCC GCC ACC GCC AGA G-3'
 RHuV_λBACK4Fv 5'-GG CGG TTG AGT CAG TAT AAC GTG CGA TCC GCC ACC GCC AGA G-3'
 RHuV_λBACK5Fv 5'-GA CGG CTG AGT CAG CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
 RHuV_λBACK6Fv 5'-TG GGG CTG AGT CAG CAT AAA ATT CGA TCC GCC ACC GCC AGA G-3'

3. Pull-through primers for introduction of restriction sites*

G. Human VH back (Sfi) primers (sense)

HuVH1aBACKSfi ——————+—FR1 heavy ——————
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG GTG CAG TCT GG-3'
 HuVH2aBACKSfi
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG AAC TTA AGG GAG TGT GG-3'
 HuVH3aBACKSfi
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTG CAG CTG GTG GAG TCT GG-3'
 HuVH4aBACKSfi
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG CAG GAG TCG GG-3'
 HuVHSuBACKSfi
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG TTG CAG TCT GC-3'
 HuVH6aBACKSfi
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTC CAG CTG CAG CAG TCA GG-3'

H. Human J kappa forward (Not) primers (anti-sense)

HuJk1FORNot ——————+—FR4 light ——————
 5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT TTC CAC CTT GGT CCC-3'
 HuJk2FORNot
 5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT CTC CAC CTT GGT CCC-3'

H. Human J kappa forward (Not) primers (anti-sense) (continued)

HuJk3FORNot ——————+—FR4 light ——————
 5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT ATC CAC TTT GGT CCC-3'
 HuJk4FORNot
 5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT GAT CTC CAC CTT GGT CCC-3'
 HuJk5FORNot
 5'-GAG TCA TTC TCG ACT TGC GGC CGC ACG TTT AAT CTC CAG TCG TGT CCC-3'

H. Human J lambda forward (Not) primers (anti-sense)

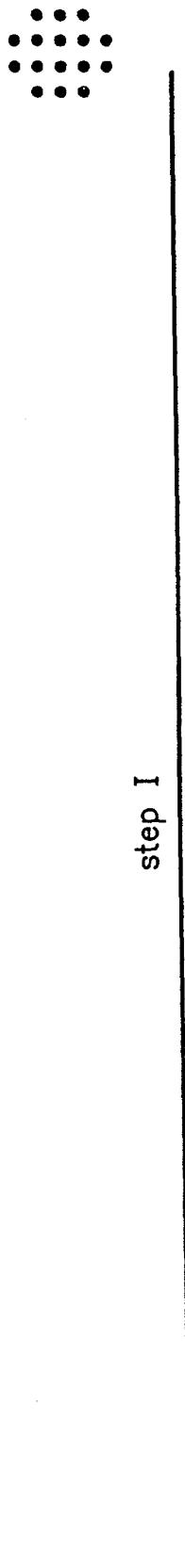
HuJ11FORNOT FR4 light
 5'-GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT GAC CTT GGT CCC-3'
 HuJ12-3FORNOT
 5'-GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT CAG CTT GGT CCC-3'
 HuJ14-5FORNOT
 5'-GAG TCA TTC TCG ACT TGC GGC CGC ACC TAA AAC GGT GAG CTG GGT CCC-3'

*Recognition site for restriction enzyme is underlined.

FIG. 13B

Title: COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUGHPUT SCREENING.

Applicant: Ault-Riche *et al.*
Serial No. 09/910,120 Filed: July 18, 2001
Our Docket No.: 25885-1751



Create 1,000 sub-libraries by separate PCR amplification
reactions using tag-specific PCR primers

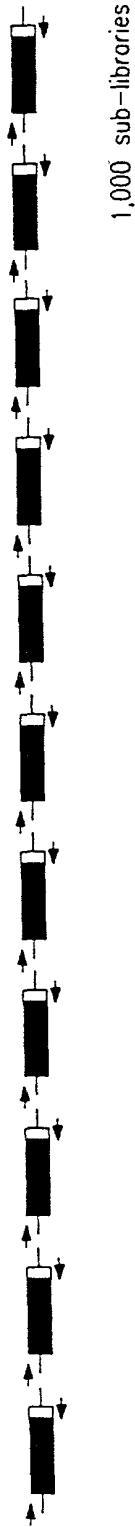


FIG. 14A

Title: COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUGHPUT SCREENING.
Applicant: Ault-Riche *et al.*
Serial No 09/910,120 Filed July 18, 2001
Our Docket No.: 25885-1751

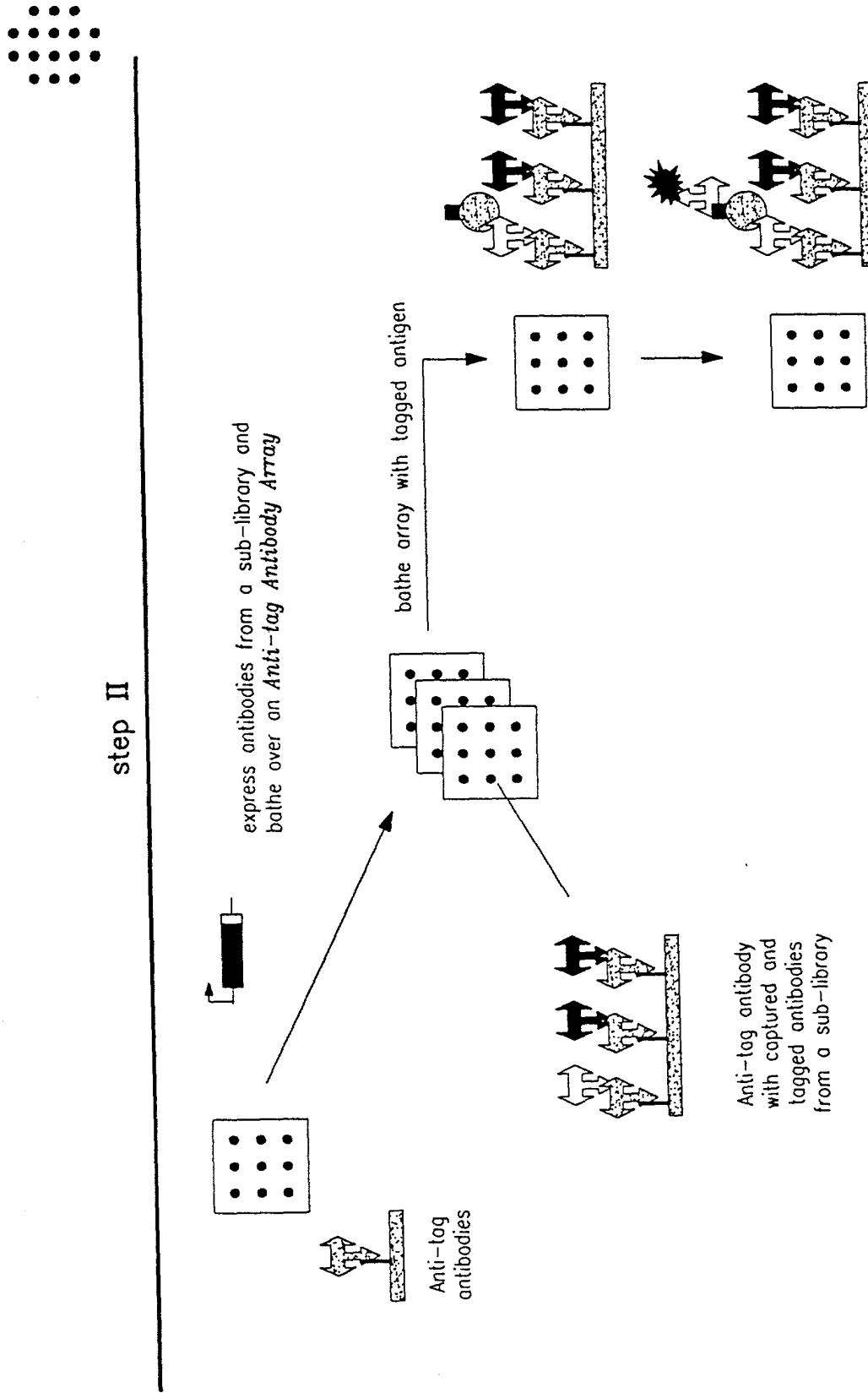
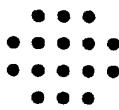


FIG. 14B

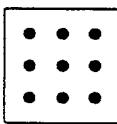
Title: COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUGHPUT SCREENING.
Applicant: Ault-Riche *et al.*
Serial No. 09/910,120 Filed: July 18, 2001
Our Docket No.: 25885-1751



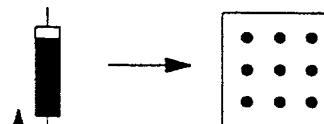
step III

Amplify the antibody genes from the identified sub-library using tag-specific PCR primers

If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have 1,000 different types of rAbs

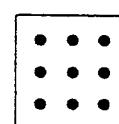


Express and purify the antibodies



Re-distribute over an Anti-tag Antibody Array

If the starting diversity of the master library was 1,000,000,000 then each spot in this array will have a single type of rAb

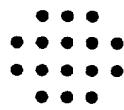


Re-survey to ID the antibody of interest!

FIG. 14C

Title: COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUHPUT SCREENING.

Applicant: Ault-Riche *et al.*
Serial No. 09/910,120 Filed: July 18, 2001
Our Docket No.: 25885-1751



summary

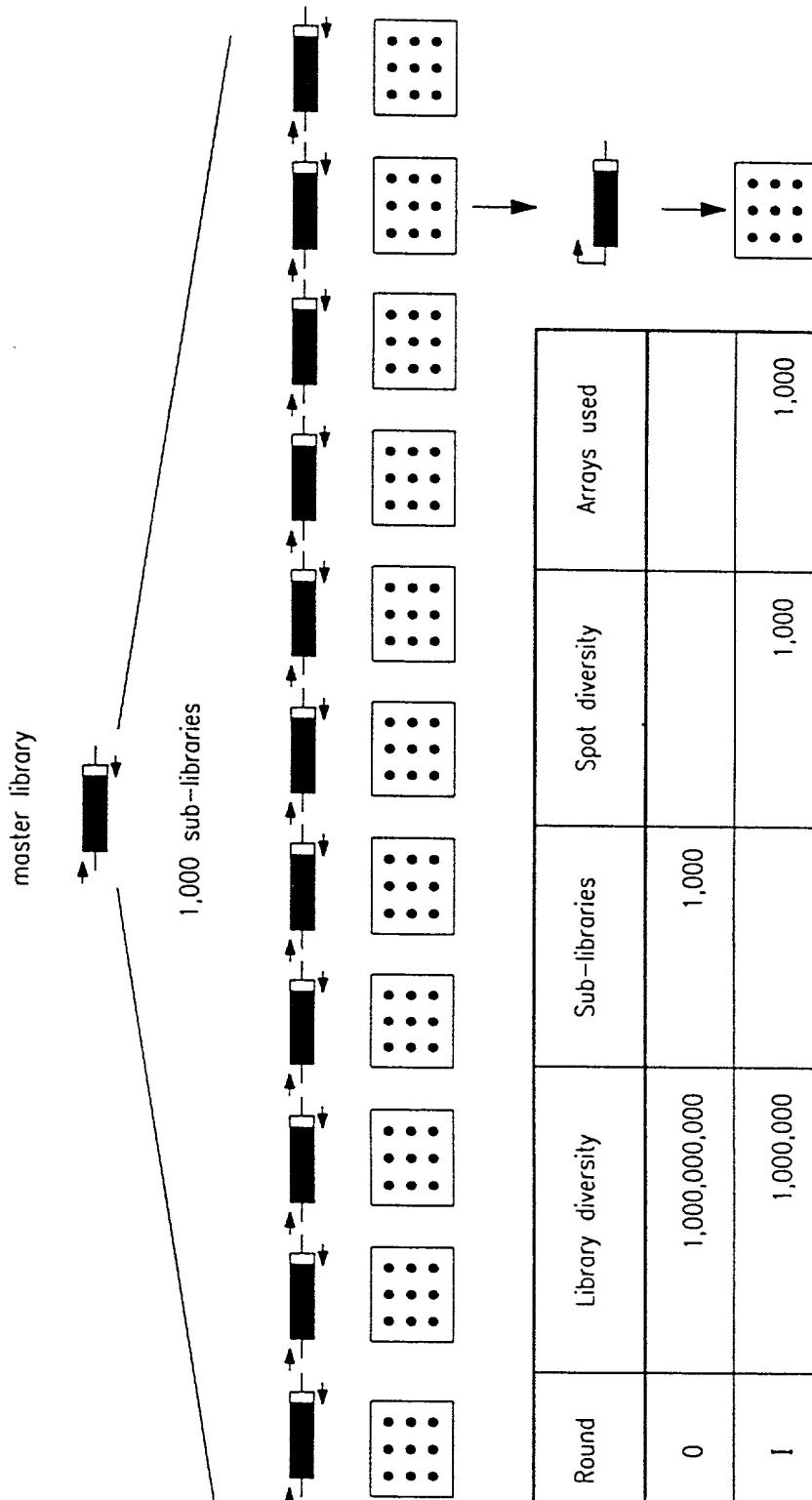
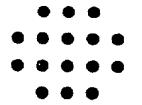


FIG. 14D

Title: COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUGHPUT SCREENING.

Applicant: Ault-Riche *et al.*
Serial No. 09/910,120 Filed: July 18, 2001
Our Docket No.: 25885-1751



Modification searches

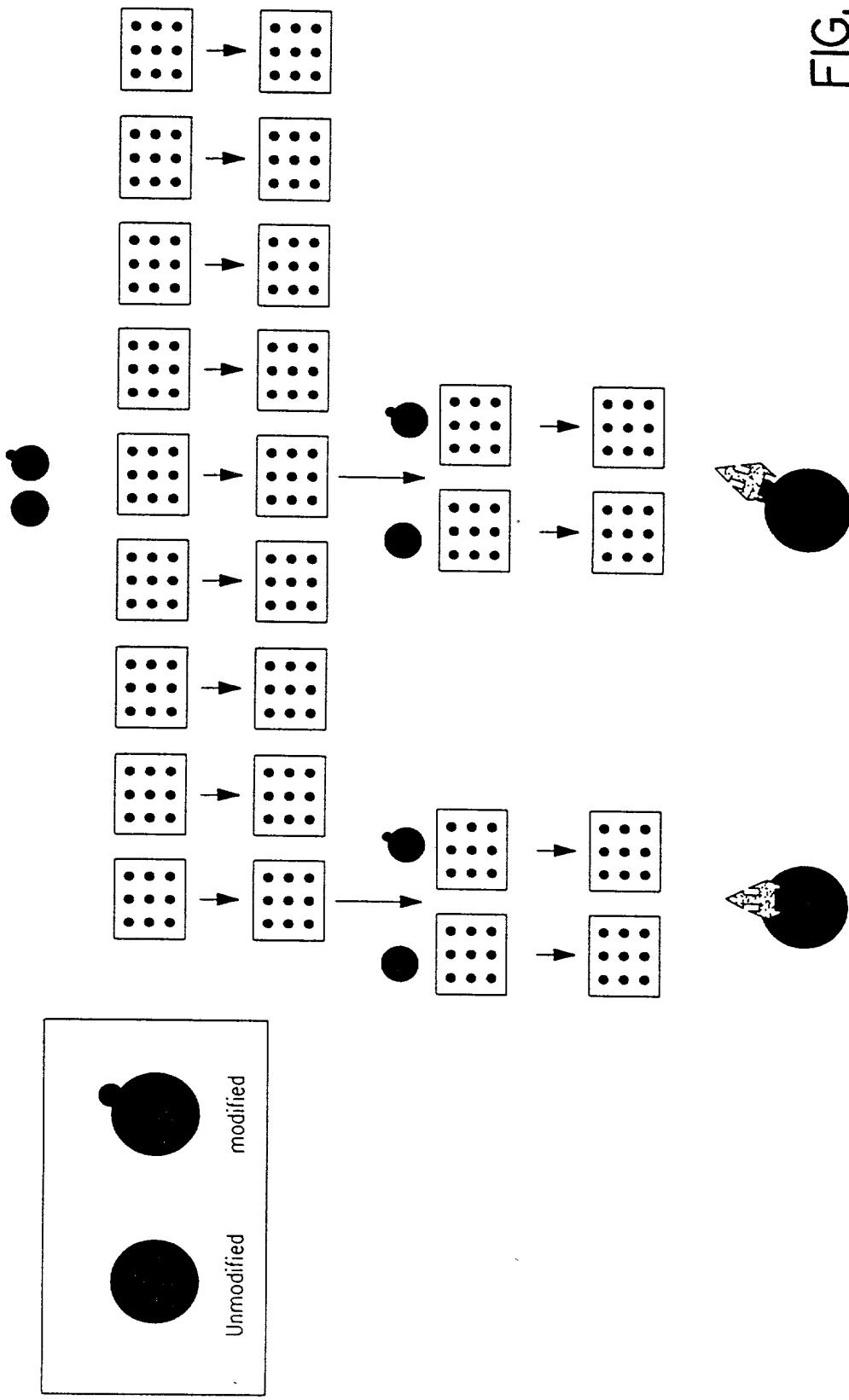


FIG. 15

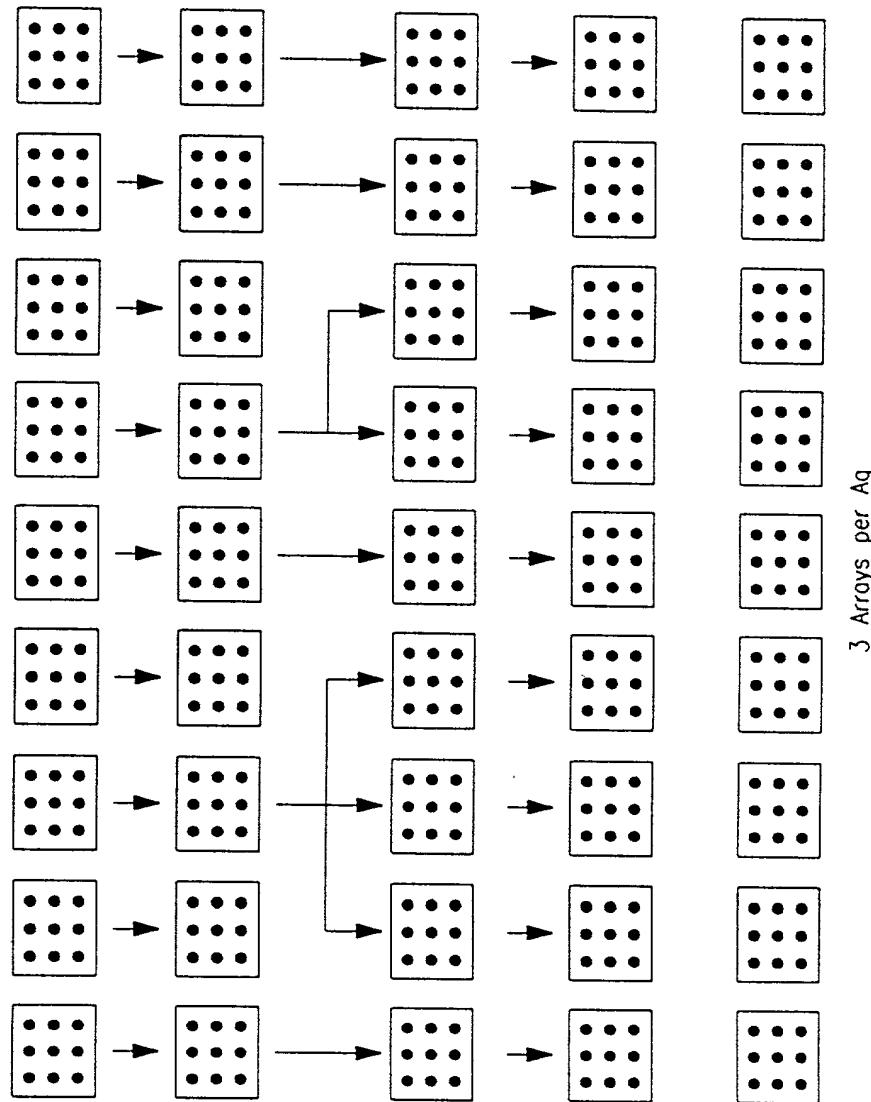
Title: COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUGHPUT SCREENING.

Applicant: Ault-Riche *et al.*
Serial No. 09/910,120 Filed: July 18, 2001
Our Docket No.: 25885-1751

Simultaneous searches

Round Arrays Bait Probe

I 1,000 Abs Ags



II 1,000 Abs Ags

III $\frac{1,000}{3,000}$ Ags Abs

FIG. 16
3 Arrays per Ag

Protein interaction mapping

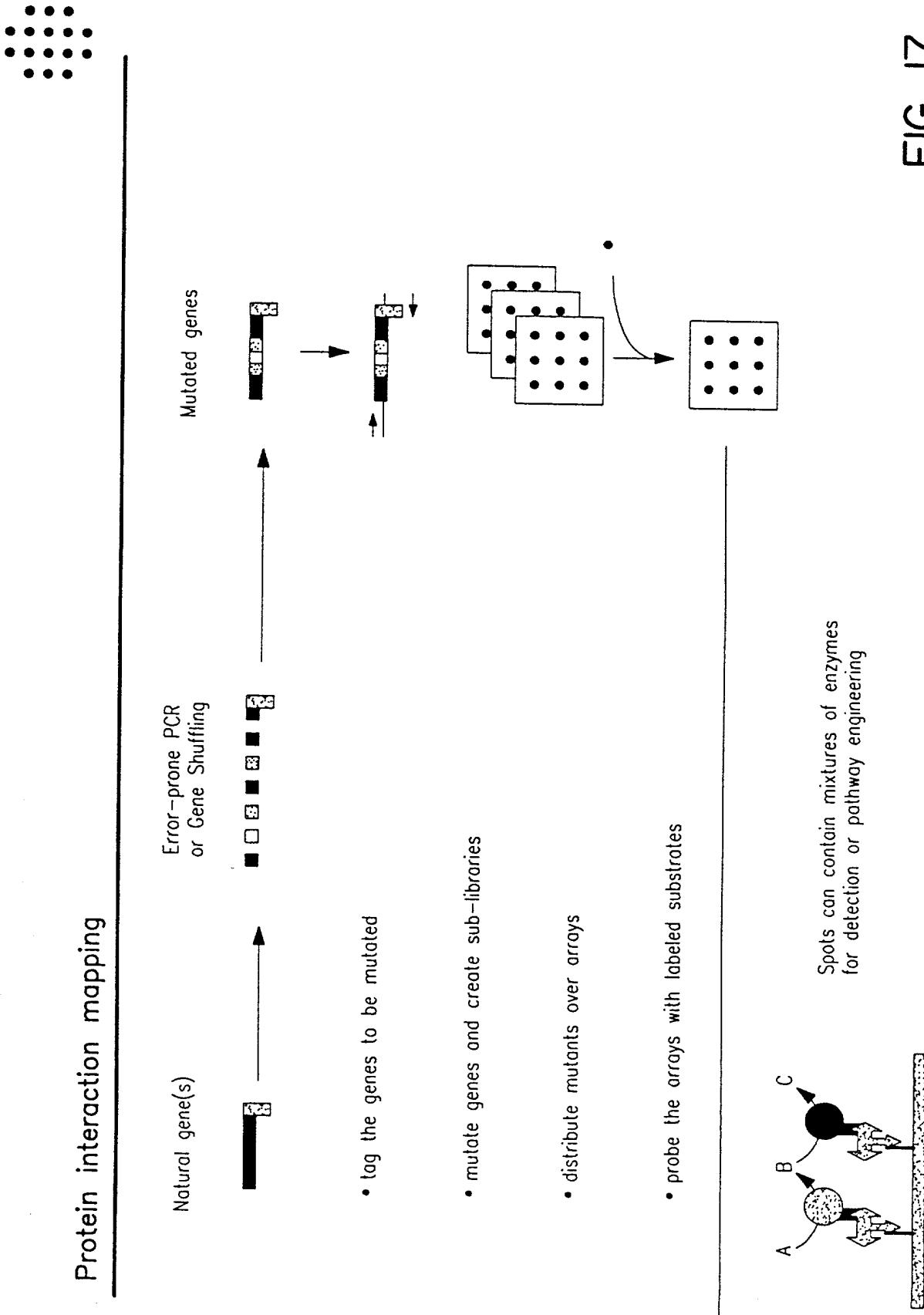


FIG. 17

Title: **COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUGHPUT SCREENING.**

Applicant: *Ault-Riche et al.*
Serial No. 09/910,120 Filed. July 18, 2001
Our Docket No. 25885-1751

Protein interaction mapping

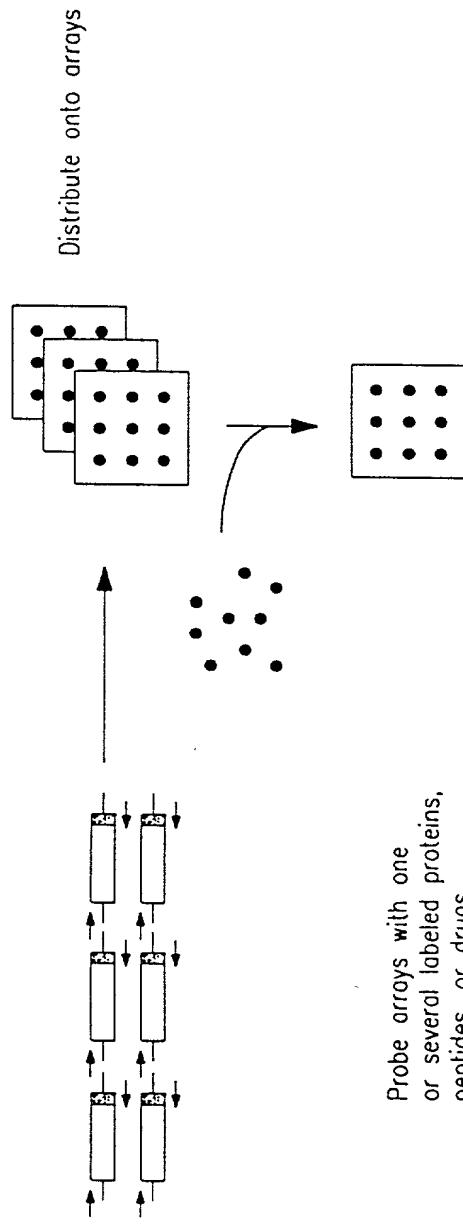
cDNA library

- Human tissue
- pathogen
- yeast

Generate a tagged cDNA library



Create sub-libraries by PCR



Probe arrays with one
or several labeled proteins,
peptides, or drugs

FIG. 18

Title: COLLECTIONS OF BINDING PROTEINS AND TAGS
AND USES THEREOF FOR NESTED SORTING AND
HIGH THROUGHPUT SCREENING

Applicant: Ault-Riche *et al.*
Serial No. 09/910,120 Filed: July 18, 2001
Our Docket No. 25885-1751

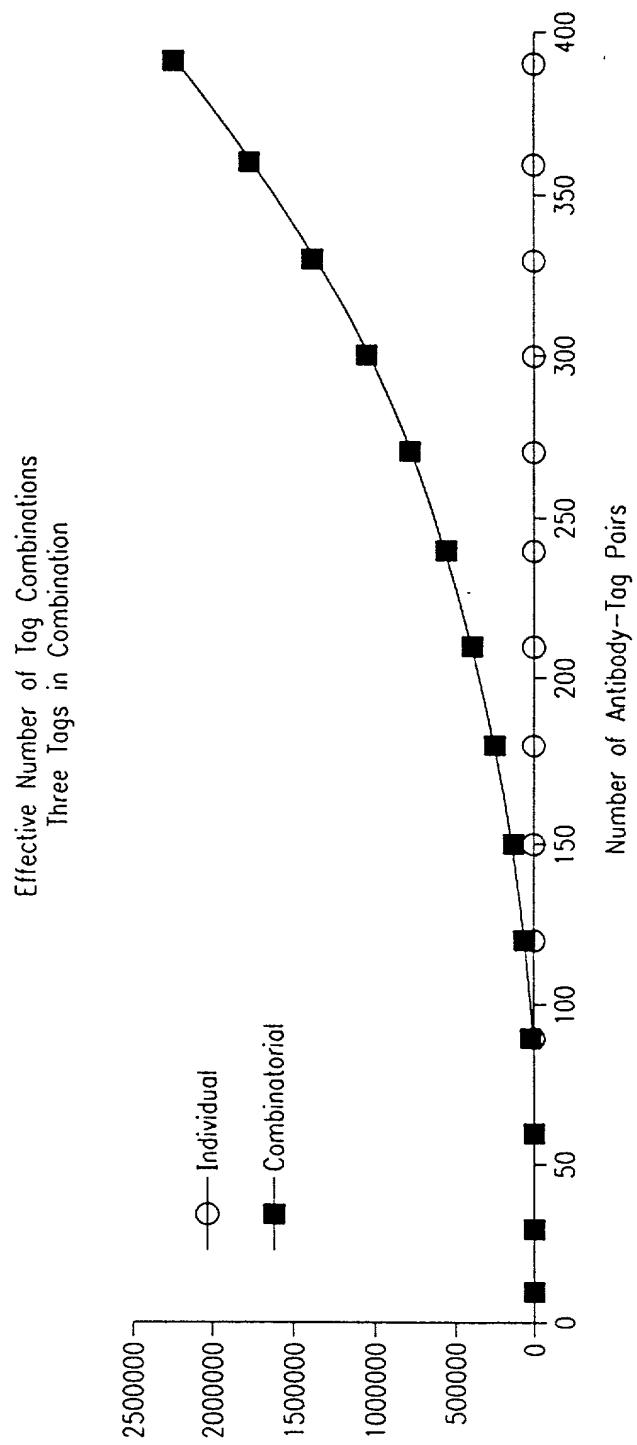


FIG. 19